Decoding the Noncoding Genome: lncRNA Dynamics and Function in Single Cells

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One of the greatest enigmas in biology today is the function of the pervasively transcribed noncoding genome. Of the numerous long noncoding RNAs (lncRNAs) found in eukaryotic cells, only a small percentage have been functionally investigated. Of those that have, many have been shown to mediate key biological processes during cellular reprograming, cell cycle progression and mammalian development, in addition to causing human cancers and neurological diseases, underscoring the need for further study of this class of molecule. Interestingly, the majority of lncRNAs are found to exist in pairs with mRNAs or other lncRNAs in both yeast and mammals, an organizational pattern that has proven functional in some cases. We are interested in further elucidating the functional relevance of lncRNAs by focusing on their gene regulatory roles and mechanisms of action within pairs. We propose to investigate the regulatory function and control mechanisms of convergently and divergently transcribed lncRNA-mRNA pairs in yeast. Based on our previous observation of mutually exclusive expression of lncRNA-mRNA pair members in single cells, we hypothesize that the physical orientation of each member of the pair in relation to its partner underlies a binary switch that is 'flipped' by environmental inputs. Specifically, these inputs may induce the expression of one member of the pair, leading to the repression of the other member, and vice-versa. To address this hypothesis, we propose to use a single-cell/single-molecule approach combined with predictive modeling and genetic gain- and loss-of-function methods to investigate the spatial-temporal dynamics of convergent and divergent lncRNA-mRNA pairs. Understanding the pattern of lncRNA-mRNA expression in single cells in response to various environmental inputs is of fundamental importance in identifying lncRNA function and control mechanisms. Our single-cell/single- molecule approach is well-suited to this task because of its ability to spatially and temporally resolve individual molecules of multiple lncRNA/mRNA species at high resolution within the same cell and correlate them to each other and to the observed phenotype. The expected outcome of our studies is an improved understanding of lncRNA function and lncRNA-mediated mechanisms of gene regulation that could not otherwise be achieved using more conventional cell population or *in vitro* studies. The positive impact of this research is the development of a novel, robust and highly informative methodology to interrogate lncRNA function and differentiate between different transcriptional models, independent of the species or specific cellular pathway, which is expected to open new avenues of research in the field.